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**The effect of buttermilk or buttermilk powder addition on functionality, textural, sensory and volatile characteristics of Cheddar- style cheese**

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**Abstract**

The influence of buttermilk or buttermilk powder addition to cheese milk or cheese curds respectively on cheese functional properties, free fatty acid profiles and subsequent volatile and sensory characteristics was investigated. Buttermilk addition to cheese milk resulted in a softer cheese compared to other cheeses, with a significantly reduced flowability, while buttermilk powder addition had no influence on cheese firmness but cheese flowability was also reduced compared to the control cheese. Larger pools of free fat, higher levels of free fatty acids, volatile compounds and significant differences in sensory profiles associated with off-flavour were also observed with the addition of buttermilk to cheese milk. Application of light microscopy, using toluidine blue stain, facilitated the visualization of fat

globule structure and distribution within the protein matrix. Addition of 10 % buttermilk powder resulted in significant increases in volatile compounds originating from proteolysis pathways associated with roasted, green aromas. Descriptive sensory evaluation indicated few differences between the 10 % buttermilk powder and the control cheese, while buttermilk cheeses scored negatively for sweaty, barnyard aromas, oxidized and off flavors, correlating with associated volatile aromas. Addition of 10 % buttermilk powder to cheese curds results in cheese comparable to the control Cheddar with some variations in volatile compounds resulting in a cheese with similar structural and sensory characteristics albeit with subtle differences in overall cheese flavor. This could be manipulated to produce cheeses of desirable quality, with potential health benefits due to increased phospholipid levels in cheese.

## 1. Introduction

Buttermilk (BM) is the aqueous side stream produced during butter production, containing the water soluble components of milk including casein, whey proteins, lactose and bioactive material originating from the milk fat globular membrane (MFGM) (Roesch, Rincon, & Corredig, 2004). BM is produced on a large scale with ~2 million tonnes produced within the E.U. in 2015 (Eurostat, 2016) and is considered a low-value product (Roesch et al., 2004). The composition of BM is similar to skim milk for levels of protein, lactose and ash content (Lambert et al., 2016; Vanderghem et al., 2010). BM is a unique by-product in that it contains high levels of valuable components such as polar lipids (1.2-2.1 % polar lipids on dry matter) (Vanderghem et al., 2010).

The potential use of buttermilk in the dairy industry has been investigated at both industrial and academic level for an extensive period of time (Dewettinck et al., 2008; Fuller,

Kuhlenschmidt, Kuhlenschmidt, Jiménez-Flores, & Donovan, 2013; Gassi et al., 2016; Govindasamy-Lucey et al., 2007; Turcot, Turgeon, & St Gelais, 2001; Vanderghem et al., 2010). The majority of buttermilk is dehydrated, due to large volumes, via evaporation and spray drying resulting in buttermilk powder (BMP). Areas in which BM products are currently utilised include the bakery sector where the addition of buttermilk powder during dough formation has been shown to improve crumb texture (Madenci & Bilgiçli, 2014) and yogurt manufacture for enhanced water binding capabilities, due to the presence of milk fat globule membrane (MFGM) material, to increase yield and improve texture characteristics (Le et al., 2011; Romeih, Abdel-Hamid, & Awad, 2014; Saffon et al., 2013; Trachoo & Mistry, 1998). MFGM material derived from BM has been proposed as a beneficial ingredient for infant formulas and other nutraceutical products due to the benefits associated with phospho- and glycolipid components (Spitsberg, 2005).

Previous work involving BM addition in cheese included the fortification of cheese milk with condensed, ultra-filtered, powdered or traditional BM's including various sources (sweet, soured and whey buttermilks) and cheese types (Pizza, Cheddar and processed) (Govindasamy-Lucey, Lin, Jaeggi, Johnson, & Lucey, 2006; Govindasamy-Lucey et al., 2007; Kifah, Layla, & Baha, 2014; Morin, Pouliot, & Britten, 2008; Sodini, Morin, Olabi, & Jiménez-Flores, 2006; Turcot, St-Gelais, & Turgeon, 2002; Turcot et al., 2001). BM addition has been shown to increase moisture levels in resulting cheese curd, most likely due to the presence of amphipolar phospholipids and denatured whey proteins, the latter of which can interact with both casein micelles and  $\kappa$ -casein resulting in casein-whey complexes which can impair syneresis and increase the rennet coagulation time (RCT) due to limited accessibility to the  $\kappa$ -casein by the coagulant.

Govindasamy-Lucey, et al., (2006) (2007) found the addition of condensed sweet cream BM decreased curd strength, lowered levels of protein and fat, increased yield and decreased flowability in pizza cheese. Morin et al. (2008) made similar conclusions relating to moisture, protein and fat, however, they found no increase in cheese yield once adjusted for moisture content. El.Sayed et al. (2010) concluded the addition of concentrated sweet BM to processed cheese spreads resulted in improved organoleptic parameters and decreased meltability as storage period increased. Turcot et al. (2002) fortified low-fat cheese milk with phospholipids in the form of concentrated BM and found increased moisture, higher primary proteolysis and bitter/ rancid flavour development as phospholipid (PL) content increased. Low-fat cheeses rich in PL had a softer texture and a granular microstructure. Current research into the effect of BMP addition to cheese milk and the effect on resultant cheese is limited. Romeih, Moe, and Skeie (2012) and Martinovic et al. (2013) investigated the fortification of cheese milk with BMP, compared to skim milk powder (SMP), on the microstructure and influence of MFGM material on the developing non-starter lactic acid bacteria (NSLAB) population respectively, of low-fat Cheddar cheese. MFGM material (provided via BMP addition to milk) was shown to have a minor influence on the development of NSLAB populations during ripening of cheese compared to the use of SMP. The addition of BMP resulted in a smoother, more homogenous protein network with smaller voids and a more uniformed distribution of fat globules within the cheese matrix compared to control cheese made with SMP addition.

The objectives of this study were to determine the effect of BMP addition to milled cheese curd at the point of salting on the hardness, meltability, free fatty acid content, volatile profile and sensory characteristics of Cheddar- style cheeses compared to cheeses made with milk fortified with BM and to control Cheddar cheeses. Light microscopy was also utilised to

determine differences in the microstructure of experimental cheeses and to observe the distribution of bacterial colonies.

## 2. Materials and methods

### 2.1 Cheese manufacture

Cheese manufacture was as per Hickey, Diehl, Nuzzo, Wilkinson, and Sheehan (Accepted manuscript, 2017). In summary, raw milk and BM were obtained from a local dairy company the day prior to manufacture. Cheese milk was standardized to 0.95:1 protein: fat ratio (Lawrence et al., 2004). BM (30 %) was combined with milk (70 %) and standardized as above using skim milk retentate (> 5 % protein) obtained via ultra-filtration (UF). Experimental cheeses are outlined below:

|                  |   |
|------------------|---|
| Control cheese:  | Cheddar cheese                              |
| BM cheese:       | Cheese milk comprising 70 % milk + 30 % BM  |
| 5 % BMP cheese:  | 5 % BMP added to curd (w/w) during salting  |
| 10 % BMP cheese: | 10 % BMP added to curd (w/w) during salting |

BMP was added with the salt, directly to the milled cheese curds, at a rate of 5 % (5 % BMP cheese) or 10 % w/w (10 % BMP cheese). Cheeses were vacuum packed and ripened at 8 °C for 180 d.

### 2.3 Cheese composition

At 14 d post manufacture, grated cheese samples were analysed for total salt (IDF, 1988), protein (IDF, 1993), moisture and fat via nuclear magnetic resonance (NMR) (Fast

Trac analysis system, CEM Microwave technology Ltd., Dublin, Ireland) (Castell-Palou et al., 2013). Cheese pH was measured by preparing a cheese slurry from 20 g of grated cheese combined with 12 g of H<sub>2</sub>O (45-55 °C) (British Standards Institution, 1976).

## 2.4 Cheese firmness

Six cheese cubes (25 mm<sup>3</sup>) were cut from each of the 4 cheese types, wrapped in tin foil (to avoid moisture loss) and stored at 4 °C overnight. Each cube was taken from the refrigerator and immediately compressed to 70% of original height at room temperature at a rate of 1 mm s<sup>-1</sup> on a texture analyser (model TA-HDI, Stable Micro Systems, Godalming, UK), equipped with a 5 mm compression plate and a 100 kg load cell (Guinee et al., 2015). Firmness ( $\sigma$  max), the force at 70% compression, was calculated from the resultant force/time curves.

## 2.5 Flowability

Flowability was assessed using the modified Schreiber method (Guinee & O'Callaghan, 2013). A disc of cheese, 4.75 cm in diameter and 4.5 mm in height, was placed on a circular glass dish and heated for 4 min at 280 °C in a convection oven (Binder FD 35, Binder GmbH, Tuttlingen, Germany). The cheese was allowed to cool to room temperature; the flow was defined as the % increase in mean diameter of the cheese disc during heating.

## 2.6 Free fatty acid analysis

Free fatty acid (FFA) extractions were performed on 10 g of grated cheese, according to the method described by De Jong and Badings (1990). The FFA extracts were aliquoted into amber glass vials and capped with Polytetrafluoroethylene (PTFE)/white silicone septa (Agilent Technologies, Little Island, Cork, Ireland). The FFA extracts were derivatised as

methyl esters as outlined by Mannion, Furey, and Kilcawley (2016) using a Sample Prep Workbench (Agilent Technologies). Fatty acid methyl esters extracts were analysed using Varian CP3800 gas chromatograph (Aquilant, Dublin, Ireland) with a CP84000 auto-sampler and flame ionisation detector (GC-FID) and a Varian 1079 injector (Aquilant). For the GC-FID analysis, 0.7  $\mu\text{L}$  was injected into a CP FFAP CB capillary column (32 m 0.25 mm 0.32  $\mu\text{m}$ ) (Agilent Technologies). Results are expressed as  $\mu\text{g mg}^{-1}$  of cheese.

## 2.7 Volatile analysis

Volatile analyses, including solid phase micro extraction (SPME) and analyses via gas chromatography mass spec (GCMS) were performed as per Bertuzzi et al., (2017). All analyses were carried out in triplicate. All analyses were carried out in triplicate.

## 2.8 Sensory analysis

### 2.8.1 Sensory affective evaluation

Twenty seven (untrained) consumers were recruited in University College Cork, Ireland. Sensory acceptance testing was conducted using these untrained assessors (Stone, Bleibaum, & Thomas, 2012a, 2012b; Stone & Sidel, 2004). Age range of assessors was 21-48 years old. Selection criteria for consumers were availability and motivation to participate on all days of the experiment and that they were Cheddar cheese consumers. Assessors used the sensory hedonic descriptors in Table 1 for four experimental Cheddar- style cheeses, presented in triplicate. Sensory analysis was carried out in panel booths conforming to international standards (International standard, 2007).

All samples were chilled and stored at 4°C until required (about 1 week). Samples were then held at refrigeration temperatures overnight (4 °C), before monadic presentation to



the naïve assessor panel at ambient temperatures ( $\sim 21^{\circ}\text{C}$ ) and coded with a randomly selected 3 digit code. Each assessor was provided with deionised water and instructed to cleanse their palates between tastings. Additionally, each assessor was asked to indicate their degree of liking on a 10 cm line scale ranging from 0 (extremely dislike) at the left to 10 (extremely like) at the right and rating subsequently scored in cm from left. The order of the presentation of all test samples was randomized to prevent first order and carryover effects and all experimental cheese samples were presented in triplicate.

### 2.8.2 Ranking descriptive analysis

Eight panellists were recruited in University College Cork, Ireland. Age range of assessors was 22- 48 years old. Selection criteria for panellists were availability and motivation to participate on all days of the experiment and that they were Cheddar cheese consumers. Panellists were trained (Richter et al. 2010) and used the sensory Intensity descriptors in Table 1 for experimental samples as described in section 2.8.1. Ranking Descriptive analysis (RDA) (Dairou and Sieffermann 2002, Richter et al. 2010) was carried out in panel booths conforming to international standards (International standard 2007) on the four Cheddar cheese samples to be tested in triplicate. All samples were stored at  $4^{\circ}\text{C}$  before being presented to the panel at ambient temperatures ( $21^{\circ}\text{C}$ ) and coded with a randomly selected 3 digit code.

The cheeses were immediately served to panellists simultaneously who ranked samples using the lexicon in Table 1 (intensity). Each assessor was provided with deionised water and instructed to cleanse their palates between tastings. Additionally, each assessor was presented with triplicate samples (over separate sessions) which reflect different production batches and asked to assess the intensity of the attributes (Table 1) according a 10 cm line

scale ranging from 0 (none) at the left to 10 (extreme) at the right and rating subsequently scored in cm from left. The order of the presentation of all test samples was randomized to prevent first order and carryover effects.

## 2.9 Light microscopy analysis

Cheese protein matrix and fat distribution were analysed via transmission light microscopy. Cheese samples were sectioned into 0.5 cm x 0.5 cm x 0.5 cm blocks using a razor blade and incubated in 1 % osmium tetroxide ( $\text{OsO}_4$ ) (Electron Microscopy Sciences, Cat. No. RT19180) in 0.1 M Sorensen's phosphate buffer (0.133 M  $\text{Na}_2\text{HPO}_4$ , 0.133 M  $\text{KH}_2\text{PO}_4$ ; pH 7.4) for 1 hour at room temperature.  $\text{OsO}_4$  was removed and samples were placed in 0.1 M Sorensen's phosphate buffer at room temperature for 10 minutes. Samples were dehydrated in 30 % ethanol (Sigma, 459836) for 10 minutes followed by dehydration in 50 %, 70 % and 90 % ethanol for 10 minutes each all at room temperature. Samples were placed in 100 % ethanol three times for 20 minutes each.

Samples were incubated twice in 1,2-Propylene oxide (Merck, Cat. No. 807027) for 15 minutes. 1,2-Propylene oxide was removed and samples were incubated in 50:50 Agar epon resin:1,2-Propylene oxide for 1 hour at room temperature. Samples were removed from the 50:50 mixture and immersed in agar epon resin for 2 hours at 37 °C and then placed into epon moulds and polymerised at 60 °C for 48 hours. Sections (0.5  $\mu\text{m}$ ) were obtained using a Leica EM UC6 ultramicrotome and stained using 0.1 % toluidine blue (Electron Microscopy Sciences, Cat. No. 22050). Samples were imaged using a 10 X/ 0.45 CFI Plan Apo Nikon

objective on a Nikon E80i transmission light microscope with a Canon EOS600D 18.0 megapixel complementary metal-oxide-semiconductor (CMOS) camera.

## 2.10 Statistical analysis

Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure (SAS, 1995) where the effects of treatments (BMP addition or use of liquid BM in cheese milk), including the standard deviation between each of the three replicates, were estimated for response variables relating to cheese composition, firmness, meltability levels of individual FFA's and both hedonic and ranking descriptive analysis. Duncan's multiple comparison tests was used as a guide for paired comparisons of the treatment means. The level of significance was determined at  $P < 0.05$  (Sheehan, Fenelon, Wilkinson, & McSweeney, 2007).

ANOVA for the split plot design was carried out using a GLM procedure (SAS, 1995). Statistically significant differences ( $P < 0.05$ ) between means were determined by Fisher's least significant difference. Application of a split plot design to determine the effects of the aforementioned treatments, ripening time and their interaction on the various parameters were performed as reported by Sheehan et al. (2007).

ANOVA-Partial Least Squares Regression (ASLPR) was used to process the raw data arising from volatile compound analysis. Statistical analysis of volatile compounds was performed using Tukey ANOVA analysis via Minitab 17 (Minitab Inc., Coventry, UK) as per Bertuzzi et al. (2017).

## 3. Results and discussion

### 3.1 Composition

Cheese composition was discussed in detail in Hickey et al. (Submitted manuscript, 2017) and in Supplementary Table 1. In summary, the addition of 10% BMP resulted in significantly higher levels of moisture, salt and subsequent salt-in-moisture (S/M) levels compared to all other cheeses. BMP addition resulted in a significant reduction ( $P < 0.05$ ) in the levels of protein compared to the control cheeses. BM addition to cheese milk resulted in significantly higher cheese moisture and moisture in non-fat substance (MNFS) levels (39.45 %, 56.07 %) compared to the control (36.15 %, 52.38 %), 5 % (36.7 %, 52.12 %) and 10 % BMP (37.74 %, 51.85 %) cheeses respectively, possibly due to higher levels of denatured whey proteins, resulting from high heat treatment of both the cream and subsequent BM, as hypothesized by Govindasamy-Lucey et al. (2006) and Morin et al. (2008). Fat levels in cheeses were significantly lower in the 10 % BMP (27.23 %) and BM cheeses (28.87 %) compared to the control cheeses (31.0 %). Fat in dry matter (FDM) levels were significantly lower ( $P < 0.05$ ) in the BMP cheeses.

### 3.2 Cheese functionality

#### 3.2.1 Cheese rheology

Cheese firmness was defined and determined as the maximum force required to compress a cheese sample by 70 % of the original height. There was a significant influence ( $P < 0.001$ ) of treatment, with BM cheeses having a softer texture (Fig. 1) compared to all other cheeses throughout ripening, which is similar to previous findings where BM has been added to cheese milk prior to curd formation (Govindasamy-Lucey et al., 2006; Turcot et al., 2002). The significantly lower  $\sigma$ -max observed in the BM cheese was most likely associated

with the significantly higher moisture level compared to all other cheeses, resulting in increased bound water associated with the *para*- casein matrix, which in turn resulted in a more porous cheese structure (Everett & Auty, 2008; McCarthy, Wilkinson, Kelly, & Guinee, 2016). The addition of BMP to the cheese curd had no significant ( $P > 0.05$ ) impact on cheese firmness, which is interesting, especially for the 10 % BMP cheeses as they had significantly higher ( $P < 0.05$ ) moisture levels which would be associated with a softer cheese texture, but this may have been counter balanced by the reduction in fat content, as reduced fat has been associated with a firmer cheese texture (Henneberry, Wilkinson, Kilcawley, Kelly, & Guinee, 2015; Sheehan & Guinee, 2004). Firmness decreased significantly ( $P < 0.05$ ), as ripening progressed, for all cheeses. The largest decrease in cheese firmness occurred between 60 to 120 d of ripening, at which point cheese firmness began to increase slightly for control, 5 % BMP and BM cheeses up until 180 d of ripening. This increase may be due to the increased incorporation of the BMP into the cheese matrix allowing the cheese curds to become increasingly fused during the latter stages of ripening resulting in a firmer cheese, more study is needed in this area to fully understand the results observed. The firmness profile for the control cheese is similar to that reported by McCarthy et al. (2016) for a similar style cheese.

### 3.2.2 Cheese flowability

Cheese flowability was determined at 180 d of ripening. There was a significant effect of treatment ( $P < 0.05$ ) with significantly lower flowability evident in the 5% BMP (39 %), 10 % BMP (36 %) and BM (39 %) cheeses compared to the control cheeses (59 %). There was no significant difference ( $P > 0.05$ ) between the BM, 5 or 10 % BMP cheeses (Fig. 2). The reduced level of flowability associated with the BM cheese was most likely due to the

reduced fat level observed in this cheese combined with higher moisture levels, which has been shown previously to negatively affect the functional properties of cooked cheese (Henneberry et al., 2015; McCarthy et al., 2016) and due to the presence of MFGM material, such as PL's, which may crosslink with proteins reducing meltability and subsequent flow (Poduval & Mistry, 1999). The presence of free fat as observed in the BM cheeses has been linked to increased flowability (Everett & Auty, 2008) but in this case it would appear that increased moisture and lower total fat levels exerted more of an influence. The reduction in flowability observed for the BMP cheeses was attributed to a combination of increased moisture content (10% BMP cheese), decreased levels of FDM (Supplementary Table 1) and primary proteolysis (results not shown), the latter of which is a key component in determining the flowability of molten cheese (McCarthy et al., 2016).

The pH of cheese is an important mediator of cheese flowability as reduced pH levels after manufacture indicate potential increases in soluble calcium lost during cheese making and subsequent increases in cheese flowability (Pastorino, Hansen, & McMahon, 2003; Sheehan & Guinee, 2004). In this study, pH levels were similar for all cheeses at 14 d of ripening, except the 10 % BMP cheeses which had a significantly higher pH (Supplementary Table 1), given the low level of magnitude in the difference in pH levels it is expected that the loss of soluble calcium was not a major factor in the reduced flowability in comparison to the levels of moisture, FDM and proteolysis.

### 3.3 Cheese microstructure/ fat distribution

Light microscopy was utilised in conjunction with the protein stain toluidine blue to visualise the curd protein and fat microstructure and also to observe bacterial populations within the cheese matrix through the stains affinity for nucleic acids (Fig 3 A-D) at 14 d of

ripening. As expected the control, 5 % BMP and 10 % BMP cheese had similar microstructures with similar patterns of fat distribution with the majority of fat present in the form of individual fat globules with areas of coalescence and free fat also evident (Fig 3 A,C,D), similar to results observed by (Turcot et al., 2002). Interestingly there was evidence of large crystals, most likely lactose crystals due to the early stage of ripening, within the matrix of the 5 % BMP cheese as seen in Fig. 3 C which were also evidence in the 10 % BMP also (results not shown) and which were noticeably absent from the control and BM cheeses. These images show high levels of free fat in the BM cheeses which may increase the possibility of greater FFA generation (Deeth, 2006). The location of bacterial populations was also evident and clearly indicated the bacterial colonies were associated with the protein/fat interface of the cheese with many colonies located within the voids once occupied by fat globules. Incorporation of BMP appeared to have no effect on cheese microstructure in relation to fat distribution and/ or the fat globule structure.

### 3.4 FFA composition

There was a significant effect of treatment ( $P < 0.05$ ) with higher levels of total free fatty acids (TFFA's) observed in the BM cheese (2857 mg/ kg<sup>-1</sup> of cheese) compared to the control (1544 mg/ kg<sup>-1</sup> of cheese), which in turn had significantly higher ( $P < 0.05$ ) concentrations of FFA's compared to the 5 % BMP (1056 mg/ kg<sup>-1</sup> of cheese) and the 10 % BMP (1049 mg/ kg<sup>-1</sup> of cheese) cheeses respectively at 180 d of ripening. Ripening time had a significant effect ( $P < 0.05$ ) on levels of FFA's in BM cheeses, with no significant difference ( $P > 0.05$ ) in levels observed in the control, 5 % and 10 % BMP cheese between 7 and 180 d of ripening (Fig. 4). This is possibly due, in part, to the loss of short chain FFA's (SCFFA) during the drying of the BMP as these highly volatile compounds have been shown

to be water soluble (Hickey *et al.* 2007) combined with lower levels of lipolytic activity from the starter cultures or potentially limited substrate availability. The majority of lactic acid bacteria (LAB) cultures have been shown to be capable of cleaving mono and di-glycerides but not triglycerides (Hickey *et al.*, 2006),. However, further study is required to fully understand the reduced FFA development in the BMP cheeses.

Levels of individual FFA's detected at 180 d of ripening are shown in Fig. 5. The BM cheeses registered significantly higher levels ( $P < 0.05$ ) for 10 of the 11 individual FFA's detected compared to all other cheeses, with C<sub>6</sub> the only exception. The levels of FFA's observed for the control cheese are similar to those reported by McCarthy *et al.* (2016). The dominant FFA's during ripening were C<sub>4</sub>, C<sub>14</sub>, C<sub>16</sub>, C<sub>18</sub> and C<sub>18:1</sub>, similar to those observed by O'Callaghan *et al.* (2017) for a similar style cheese at a similar point of ripening. Levels of long chain FA's (LCFFA) C<sub>16</sub> and C<sub>18:1</sub> were two times higher in BM cheeses compared to all other cheeses, however, these FA's are not the main contributors to overall cheese flavour (Atasoy & Türkoğlu, 2008). The disproportionately high levels of LCFFA's may be due to the loss of the water soluble SCFFA (C<sub>6</sub>-C<sub>10</sub>) to the cheese whey during drainage as mentioned previously (Hickey *et al.*, 2007). Hickey, Kilcawley, Beresford, and Wilkinson (2006) indicated that starter bacteria are the main agents of lipolysis in pasteurised Cheddar cheese.

Increased FFA concentrations observed in the BM cheese may be due to the introduction of free fat from the pasteurisation of the cream and the subsequent churning process, which combined with further pasteurisation steps applied to the resulting buttermilk may lead to the destruction of the milk fat globular membrane (MFGM). This may increase the levels of free fat, as previously reported when butteroil was added to cheese milk (Law, Sharpe, Chapman, & Reiter, 1973; Morin, Jiménez-Flores, & Pouliot, 2007). The combination of high temperature processing and bacterial activity may allow for increased



rates of oxidation and degradation of the triglycerides by lipolytic enzymes (Hickey et al., 2006).

### 3.5 Volatile analysis

Volatile compounds in cheese arise from lipolysis, glycolysis and proteolysis pathways which are mediated by the starter and NSLAB bacteria in the ripening cheese (Kilcawley, 2017). In this study, 37 volatile compounds were detected in the experimental cheeses which included 8 ketones, 7 alcohols, 5 aldehydes, 5 acids, 3 sulphurs, 2 esters, 2 benzene compounds, 2 pyrazine compounds, 2 terpenes and one lactone. While the majority of these volatile compounds were detected in all cheeses, those compounds which were significantly influenced by the treatments used are listed, with their associated aromas, in Table. 2. There were significant differences between the volatile characteristics of the experimental cheeses as seen in the partial least square regression (PLSR) analysis (Fig. 6) where the control, 10 % BMP and BM cheeses are significantly different ( $P < 0.05$ ) from each other as indicated by their differential positioning on the PLSR plot. The control cheese did not significantly influence the formation of any volatile compounds in comparison to the experimental cheeses. All compounds found in the control cheese were also found in the BMP and BM cheeses, suggesting the addition of BM or BMP resulted in the variations in volatile compounds observed.

The 12 compounds significantly influenced by BMP or BM addition included 4 aldehydes, 4 acids, 1 alcohol, 1 sulphur compound, 2 pyrazine compounds and a lactone compound. Addition of BM to cheesemilk significantly influenced the levels of nonanal, butanoic, hexanoic and octanoic acids along with  $\sigma$ - Decalactone all of which are associated with sweaty, musty, rancid, milk fat aromas. Nonanal is a product of lipid oxidation resulting

from the further breakdown of oleic acid which is found in high levels in milk fat (Lindmark, 2008, Torkamani et al., 2014). Lactones are cyclic compounds, the precursors of which are hydroxylated fatty acids derived from, in this case, milk fat (Kilcawley, 2017). The acids are all indicative of lipolysis and increased levels of FFA's (Fig. 5) as observed in this study for the BM cheeses (Moio & Addeo, 1998).

One compound was positively associated with both the 5 % and 10 % BMP cheeses; Benzenacetaldehyde (also known as Phenylacetaldehyde) has a honey-like, green aroma and is associated with phenylalanine reduction (Yvon and Rijnen, 2001), but both the 5 and 10 % BMP cheeses recorded significantly lower levels of phenylalanine compared to the control and BM cheeses (Supplementary Table 2), suggesting the compound may arise from lactose metabolism by LAB (McSweeney & Sousa, 2000; Oumer et al., 2001; Wu & Liou, 1992). The addition of 10 % BMP to the cheese curd positively influenced the formation of hexanol, 2- methyl propanal, 2- methyl butanal, methional, 2,5- dimethylpyrazine and trimethylpyrazine compounds, all of which have been associated previously with proteolytic activity and Maillard reactions (Chen, Song, & Ma, 2009; Kilcawley, 2017; Wu & Liou, 1992), except for hexanol which is the product of secondary oxidation of lipids, but has a flowery, green aroma (Mortensen, Sørensen, Danielsen, & Stapelfeldt, 2003). The compounds 2- methyl propanal and 2-methyl butanal are associated with the degradation of branched-chain amino acids due to the activity of *L. lactis* bacteria and associated with a malty, almond like aroma (McSweeney & Sousa, 2000), while methional is associated with the further catabolism of the amino acid methionine and presents a baked potato type aroma (McSweeney & Sousa, 2000). The pyrazine compounds associated with the 10 % BMP cheese have been linked to Maillard reactions due to heating (Alasalvar, Shahidi, & Cadwallader, 2003), in this case this is most likely the result of multiple heat treatments, evaporation and spray drying of the BMP (Mahajan, Goddik, & Qian, 2004), especially in the

case of 2,5-dimethylpyrazine as this compound was only detected in cheeses containing BMP.

It is also known that fatty acid composition is significantly influenced by the upstream diet of the animal, the milk of which, is further processed to eventually produce buttermilk and buttermilk powder (Kristensen, Kröger-Ohlsen, & Skibsted, 2002; Urbach, 1990). The resulting BM and BMP may differ significantly in fatty acid composition and subsequent volatile compound profiles depending on the diet of the animal which is often season dependent. The addition of 10 % BMP generated specific volatile compounds which were associated with malty, roasted, green type aromas which are generally viewed as pleasant aromas in food matrices including cheese.

### 3.6 Sensory characteristics

Results relating to sensory hedonics and RDA at 180 d of ripening are shown in Table 3. Results indicated a significantly higher ( $P < 0.05$ ) liking for the control and 10 % BMP cheeses in relation to aroma compared to the 5 % BMP and BM cheeses. The control cheese scored significantly higher ( $P < 0.05$ ) for liking of flavour and texture compared to the 5 % BMP cheese and also scored significantly higher for liking of appearance compared to the BM cheeses while the 10 % BMP scored similar in liking of flavour, texture and aroma to the control cheeses (Table 3). However, the control cheese had a significantly higher overall acceptability compared to all other cheeses with no differences amongst the 5 % BMP, 10 % BMP and BM cheeses.

RDA is a useful method for analysing the sensory characteristics of dairy food with significant work performed in this area in recent years (Gaze et al., 2015; Janiaski et al.,

2016). RDA analysis indicated the BM cheeses scored significantly ( $P < 0.05$ ) higher for sweaty/sour aroma compared to the control and 10 % BMP cheeses and significantly higher for barnyard aroma compared to the 10 % BMP cheeses (Table 3), which is in agreement with volatile analysis discussed in section 3.4 where higher levels of compounds were detected in the BM cheese with associated sweaty, rancid, fat like aromas. Significantly higher levels of C<sub>4</sub> (Butyric acid) detected in the BM cheese, even at low concentrations, would add to the sweaty, rancid aroma of the cheese (Kilcawley, 2017). There was a significant effect ( $P < 0.05$ ) observed where BM was incorporated into the cheese milk, which resulted in a less firm texture in the mouth compared to all other cheeses, which is in agreement with texture analysis (section 3.1) where BM cheeses had a softer texture throughout ripening. Reduced firmness in the mouth may be due to increased moisture levels and possibly due to increased pools of free fat within the cheese matrix which may reduce the firmness also (Everett & Auty, 2008). There was a significant effect ( $P < 0.05$ ) of BMP addition with a less firm texture in the mouth reported in comparison to the control cheeses. The 10 % BMP cheese scored highest for crumbly texture, significantly ( $P < 0.05$ ) higher than the control and BM cheeses suggesting that BMP addition at levels  $\leq 5$  % w/w may result in a less crumbly, more pasty texture.

Interestingly, the 10 % BMP cheese scored significantly higher for sweet taste compared to the BM cheese, but no difference was observed between any of the cheeses in relation to salty taste. This is interesting as the 10 % BMP cheese had a significantly higher level of salt (2.4 % salt) compared to the other cheeses (~1.8 % salt), indicating that the sweetness, possibly attributed to the lactose within the BMP, is capable of suppressing the salty taste in the cheese (Keast & Breslin, 2003). The opposite was true for the BM cheeses, which scored significantly lower for sweet taste and numerically scored the highest for salty taste, again indicating an effect of sweet compound concentration on salty taste perception.

The BM cheese scored significantly ( $P < 0.05$ ) higher for sour, bitter tastes and off flavours compared to all other cheeses and significantly higher for oxidised flavours compared to control cheeses. This is in agreement with FFA and volatile analysis which indicated significantly higher concentrations of FFA's and subsequent association with sour and bitter volatile compounds resulting from lipid degradation, as discussed in sections 3.4 and 3.5 respectively. The 10 % BMP cheese scored significantly lower ( $P < 0.05$ ) for cream and Cheddar flavours in comparison to the control cheeses, while the 5 % BMP and BM cheeses scored significantly lower ( $P < 0.05$ ) for dairy sweet and fruity estery flavours compared to control and 10 % BMP cheeses.

Results from the independent sensory evaluation would suggest that the addition of BMP had a significant impact on sensory acceptability at both 5 and 10 % BMP while specific sensory attributes and flavour perceptions may be influenced by the weight/proportion of BMP added. The addition of 10 % BMP resulted in no significant ( $P > 0.05$ ) change in relation to the control cheeses for liking of appearance, aroma, flavour and texture, and for levels of dairy sweet flavour. The BMP cheeses scored lower for sour, bitter tastes and overall off flavours compared to the BM cheeses indicating BMP addition to cheese curd impacted significantly less on the development of off-flavours associated with Cheddar style cheeses. Addition of BMP at levels  $\geq 10$  % may give the advantage of increased levels of bioactive compounds (Phospholipids) and many favourable flavour and aroma compounds, which although may not be typical of Cheddar style cheese, may offer the opportunity to diversify flavour profiles and lead to the development of new cheese types.

#### 4 Conclusions

This study aimed to determine the effect of BMP addition to Cheddar- type cheese curds on the subsequent cheese rheology, functional properties, levels of FFA's and volatile compounds, as well as sensory properties of the resulting cheeses. BM addition resulted in a softer cheese with higher levels of FFA's. Sensory analysis of the BM cheeses indicated increased association with negative attributes such as sweaty/sour, barnyard aromas and oxidized and overall off flavours in comparison to the control cheeses, correlating with associated volatile compounds, all of which possessed sweaty, rancid type aromas. .

The addition of BMP to cheese curd, especially at 10 % w/w, resulted in a similar cheese texture but reduced flowability and reduced levels of TFFA's compared to the control cheese. There were similarities in a number of sensory attributes and a slight variation in volatile compound composition compared to the increased sweaty, rancid, off-flavours associated with the addition of BM to the cheese milk.

These results suggest that the use of BMP offers a more favourable option to boost the PL levels in cheese in comparison to BM addition, with its associated negative attributes such as decreased firmness, increased FFA profile, elevated undesirable volatile compounds and negative sensory attributes. The results of this study are of extreme relevance to an economically concious industry with a vastly expanding milk pool and a dramatic need to create innovative products aimed at the healthy cheese market in particular. This approach offers the potential to increase the utilization of a low-value, storage stable, dairy by-product (BMP) for the development of new cheese types similar to Cheddar but with slightly different sensory characteristics and with the health benefits associated with increased levels of bioactive compounds such as phospholipids.

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ACCEPTED MANUSCRIPT

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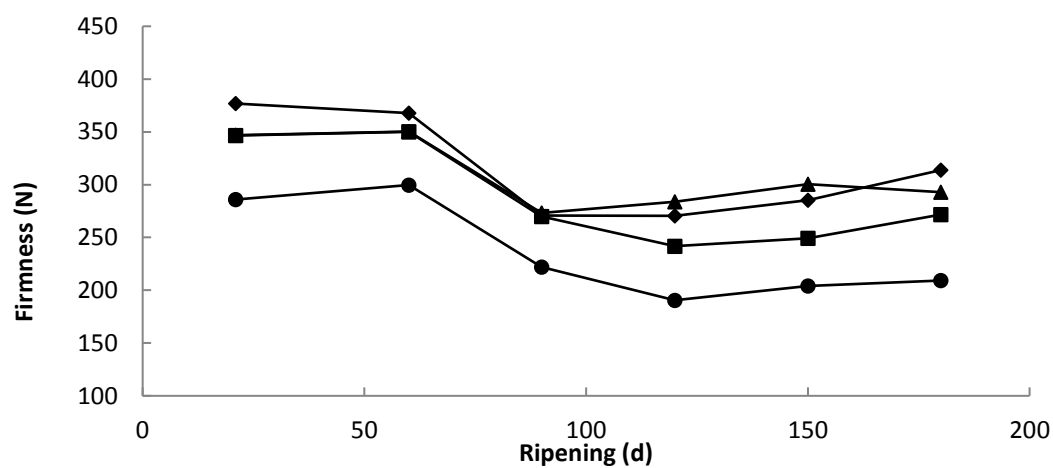


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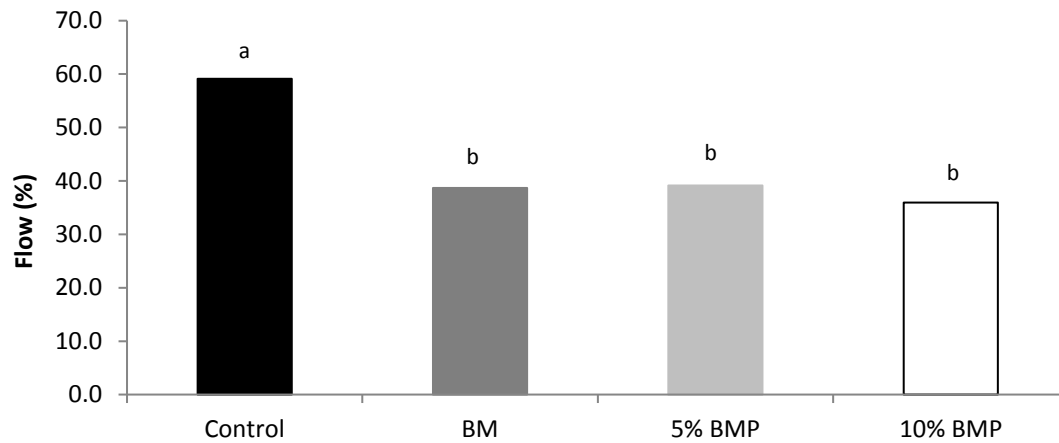
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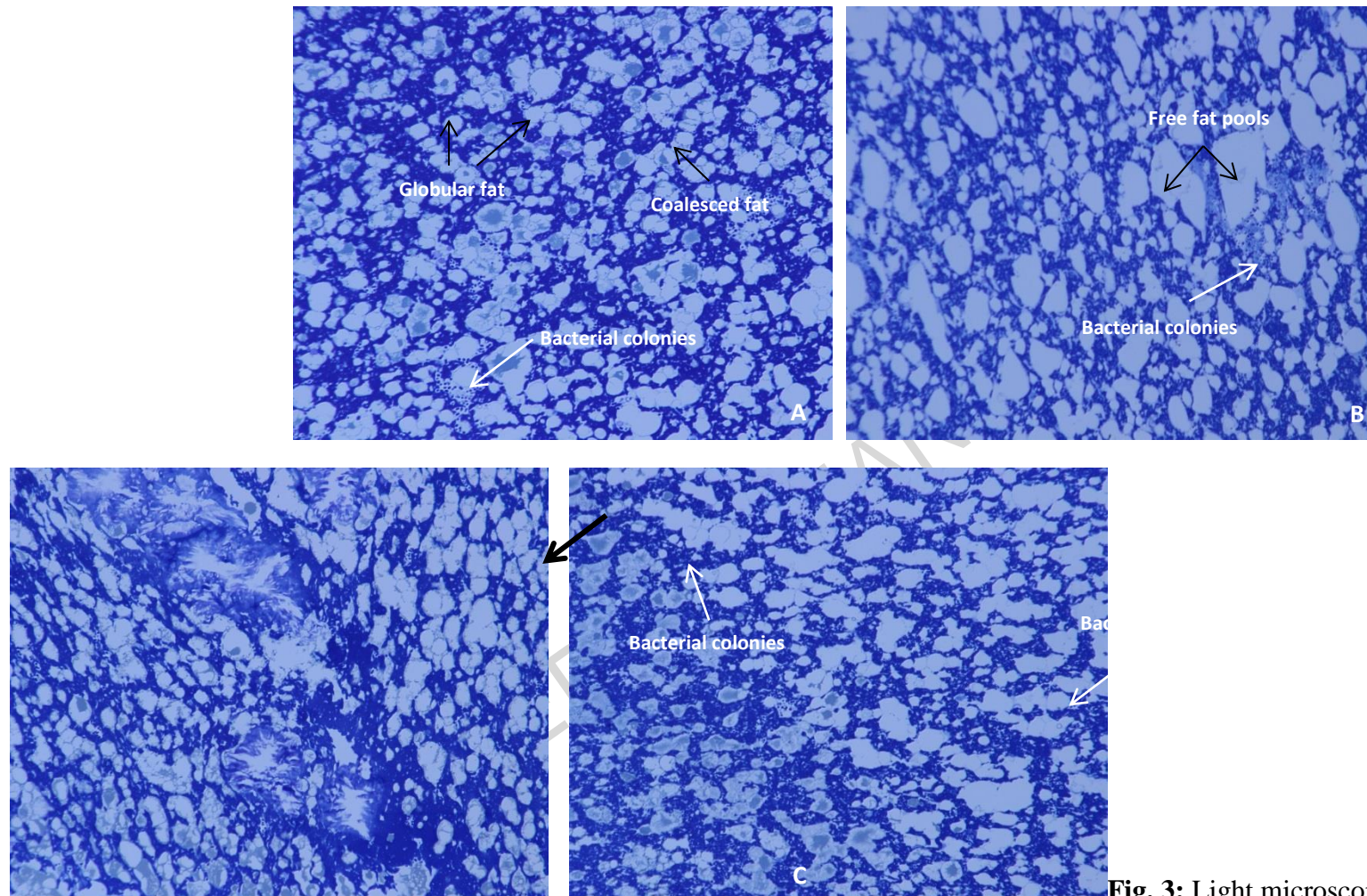
## Figures



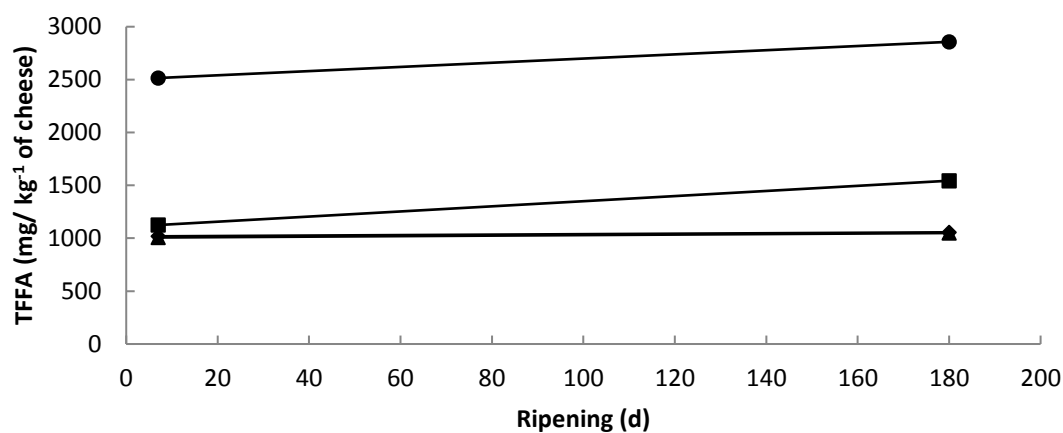
**Fig. 1:** Change in firmness ( $\delta$  max) of control (■), BM (●), 5 % BMP (◆) and 10 % BMP (▲) cheeses over 180 d ripening. Statistical significance via GLM based split-plot analysis. Values presented are the means of three replicate trials.



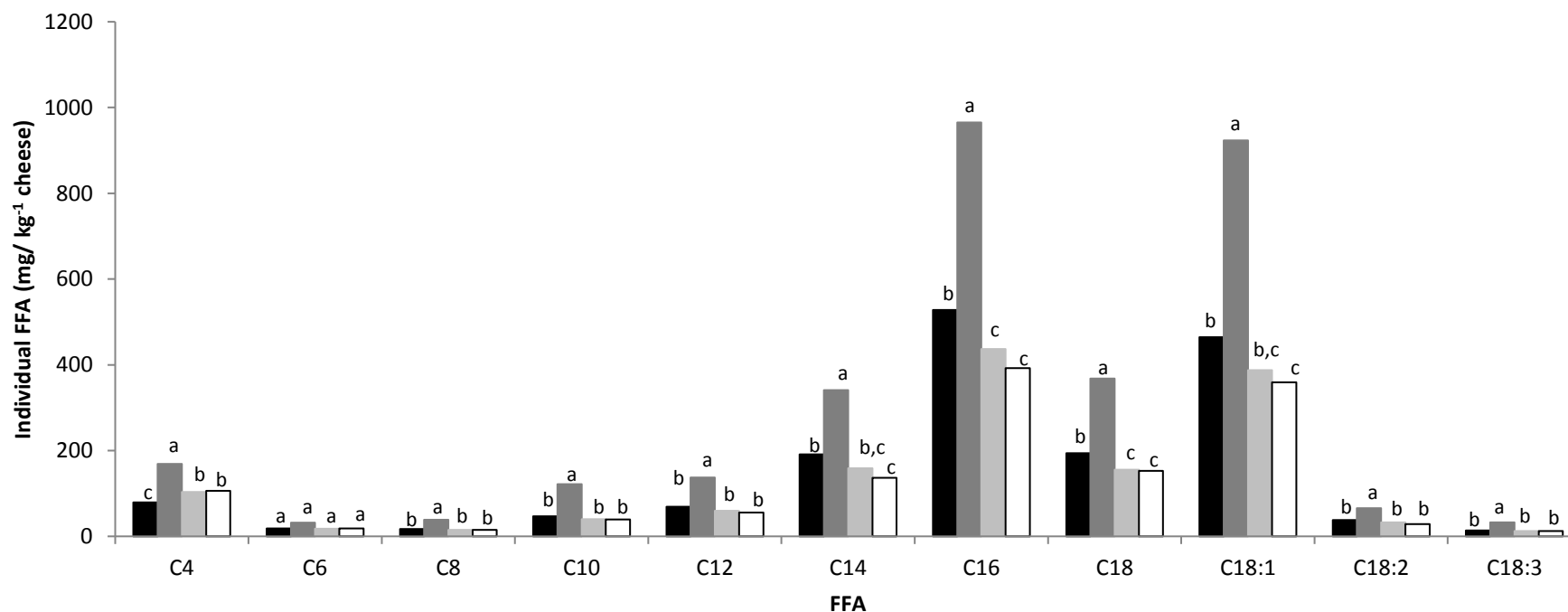
**Fig. 2:** Level of cheese flowability on heating of control (■), BM (■), 5 % BMP (■) and 10 % BMP (□) cheeses at 180 d of ripening. Letters (a-b) denote significant ( $P < 0.05$ ) differences amongst individual series (Tukey based ANOVA analysis). Values presented are the means of three replicate trials.



**Fig. 3:** Light microscopy images (10x/ 0.45 objective) of protein (blue), fat (grey) and bacterial colony (white arrows) distribution for control (A), BM (B), 5 % BMP (C) and 10 % BMP (D) cheeses at 14 d of ripening. Lactose crystals (large black arrow), Free fat pools (thin black arrow).

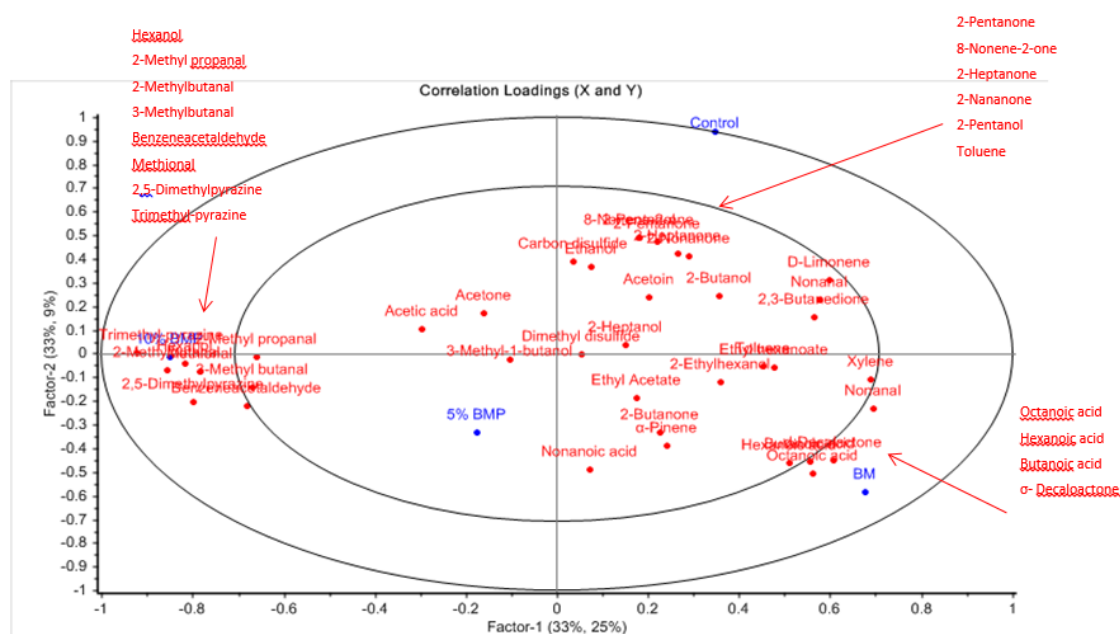


**Fig. 4:** Levels of TFFA's in control (■ ), BM (●), 5 % BMP (◆) and 10 % BMP (▲ ) cheeses over 180 d ripening. Values presented are the means of three replicate trials. Statistical significance via GLM based split-plot analysis.



**Fig. 5:** Levels of FFA's detected in the control (■), BM (■), 5 % BMP (■) and 10 % BMP (□) cheeses at 180 d of ripening. Letters (a-c) denote significant ( $P < 0.05$ ) differences between individual treatments. Values presented are the means of three replicate trials. Significance determine via Tukey based ANOVA analysis.





**Fig. 6:** Partial Least Squares Regression (PLSR) bi-plot of Control, BM, 5 % BMP and 10 % BMP cheeses and relative associations with volatiles components detected via GC/MS at 180 d of ripening. Results are the mean of three replicate trials. Statistical generation via unscramble software and one-way Tukey based ANOVA analysis.

**Table 1:** Sensory terms for the affective and descriptive evaluation of Cheddar- type cheese.

| Attribute                    | Definition  | Scale   |
|------------------------------|---|---|
| <i>Hedonic</i>               |   |   |
| <i>Appearance-Liking</i>     | The liking of appearance  | 0 = extremely dislike10 = extremely like            |
| <i>Flavour-Liking</i>        | The liking of flavour   | 0 = extremely dislike10 = extremely like            |
| <i>Aroma-Liking</i>          | The liking of aroma   | 0 = extremely dislike10 = extremely like            |
| <i>Texture-Liking</i>        | The liking of appearance  | 0 = extremely dislike10 = extremely like            |
| <i>Overall acceptability</i> | The acceptability of the product  | 0 = extremely unacceptable10 = extremely acceptable |
| <i>Intensity</i>             |   |   |
| <i>Appearance-colour</i>     | Appearance-Ivory to orange colour   | 0 = Ivory 10 = Orange                               |
| <i>Creamy aroma</i>          | The smell associated with creamy/milky products   | 0 = none, 10 = extreme                              |
| <i>Oxidised aroma</i>        | The smell associated with oxidised dairy products   | 0 = none, 10 = extreme                              |
| <i>Barnyard aroma</i>        | The smell associated with the farm, barnyard, ox tail   | 0 = none, 10 = extreme                              |
| <i>Sweaty/sour aroma</i>     | The aromatics reminiscent of perspiration, foot odour. Sour, stale, slightly cheesy, moist, stained or odorous with sweat | 0 = none, 10 = extreme                              |
| <i>Firmness in the mouth</i> | Firm texture in the mouth   | 0 = none, 10 = extreme                              |
| <i>Crumbly</i>               | Crumbly texture in the mouth  | 0 = none, 10 = extreme                              |
| <i>Pasty</i>                 | Pasty texture in the mouth  | 0 = none, 10 = extreme                              |
| <i>Sweet taste</i>           | Fundamental taste sensation of which sucrose is typical   | 0 = none, 10 = extreme                              |
| <i>Salt taste</i>            | Fundamental taste sensation of which sodium chloride is typical   | 0 = none, 10 = extreme                              |
| <i>Sour</i>                  | Fundamental taste sensation of which lactic acid is typical   | 0 = none, 10 = extreme                              |
| <i>Bitter taste</i>          | Fundamental taste sensation of which caffeine or quinine in soda water is typical   | 0 = none, 10 = extreme                              |
| <i>Cheddar flavour</i>       | Intensity of Cheddar cheese flavour   | 0 = none, 10 = extreme                              |
| <i>Cream flavour</i>         | The flavour associated with creamy/milky products   | 0 = none, 10 = extreme                              |
| <i>Dairy sweet flavour</i>   | The flavours associated with sweetened cultured dairy products such as fruit yoghurt                                      | 0 = none, 10 = extreme                              |
| <i>Dairy fat flavour</i>     | Intensity of fat flavour  | 0 = none, 10 = extreme                              |
| <i>Off-flavour</i>           | Off-flavour (Rancid)  | 0 = none, 10 = extreme                              |
| <i>Oxidised flavour</i>      | The flavour associated with rancid or oxidised products   | 0 = none, 10 = extreme                              |
| <i>Barnyard flavour</i>      | The flavour associated with the farm, barnyard, ox tail   | 0 = none, 10 = extreme                              |
| <i>Fruity/Estery flavour</i> | The flavours associated with fatty acid ethyl esters  | 0 = none, 10 = extreme                              |

**Table 2:** Volatile compounds significantly influenced by experimental cheese treatments and relative aroma notes

| Volatile compound                  | CAS-No.    | Aroma Note   |
|------------------------------------|------------|--|
| <b>Alcohols</b>                    |            |  |
| <sup>1</sup> Hexanol <sup>d</sup>  | 111-27-3   | Resin, flower, green (Xiang, et al., 2017)                                       |
| <b>Aldehydes</b>                   |            |  |
| 2-Methyl propanal <sup>d</sup>     | 78-84-2    | Pungent, malt, green (Xiang et al., 2017)  |
| 2-Methylbutanal <sup>d</sup>       | 96-17-3    | Malty, dark chocolate, almond, cocoa (Bertuzzi et al., 2017; Xiang et al., 2017) |
| Benzeneacetaldehyde <sup>c,d</sup> | 122-78-1   | Honey-like, rose, hyacinth, green (Bertuzzi et al., 2017)                        |
| Nonanal <sup>b</sup>               | 143-08-8   | Fat, citrus, green (Xiang et al., 2017)  |
| <b>Acids</b>                       |            |  |
| Butanoic acid <sup>b</sup>         | 107-92-6   | Sweaty, butter, cheese, strong, acid (Kilcawley, 2017)                           |
| Hexanoic acid <sup>b</sup>         | 142-62-1   | Musty, piquant (Ali et al., 2017)  |
| Octanoic acid <sup>b</sup>         | 124-07-2   | Cheesy, rancid, pungent, sweat (Kilcawley, 2017)                                 |
| <b>Sulphurs</b>                    |            |  |
| Methional <sup>d</sup>             | 3268-49-3  | Baked potato, Orange (Carpino et al., 2004; Xiang et al., 2017)                  |
| <b>Pyrazines</b>                   |            |  |
| 2,5-Dimethylpyrazine <sup>d</sup>  | 123-32-0   | Roasted, popcorn (Xie, Sun, Zheng, & Wang, 2008)                                 |
| Trimethyl-pyrazine <sup>d</sup>    | 14667-55-1 | Baked, raw potato (Kilcawley, 2017)  |
| <b>Lactones</b>                    |            |  |
| σ- Decalactone <sup>b</sup>        | 705-86-2   | Coconut-like, peachy, creamy, milk fat (Kilcawley, 2017)                         |

<sup>1</sup>(a-d) denote that volatile compounds are significantly different ( $P < 0.05$ ) and positively correlated to the use of individual treatments: Control cheese (a); BM cheese (b); 5 % BMP cheese (c); 10 % BMP cheese (d). Significance based on Tukey based ANOVA analysis.

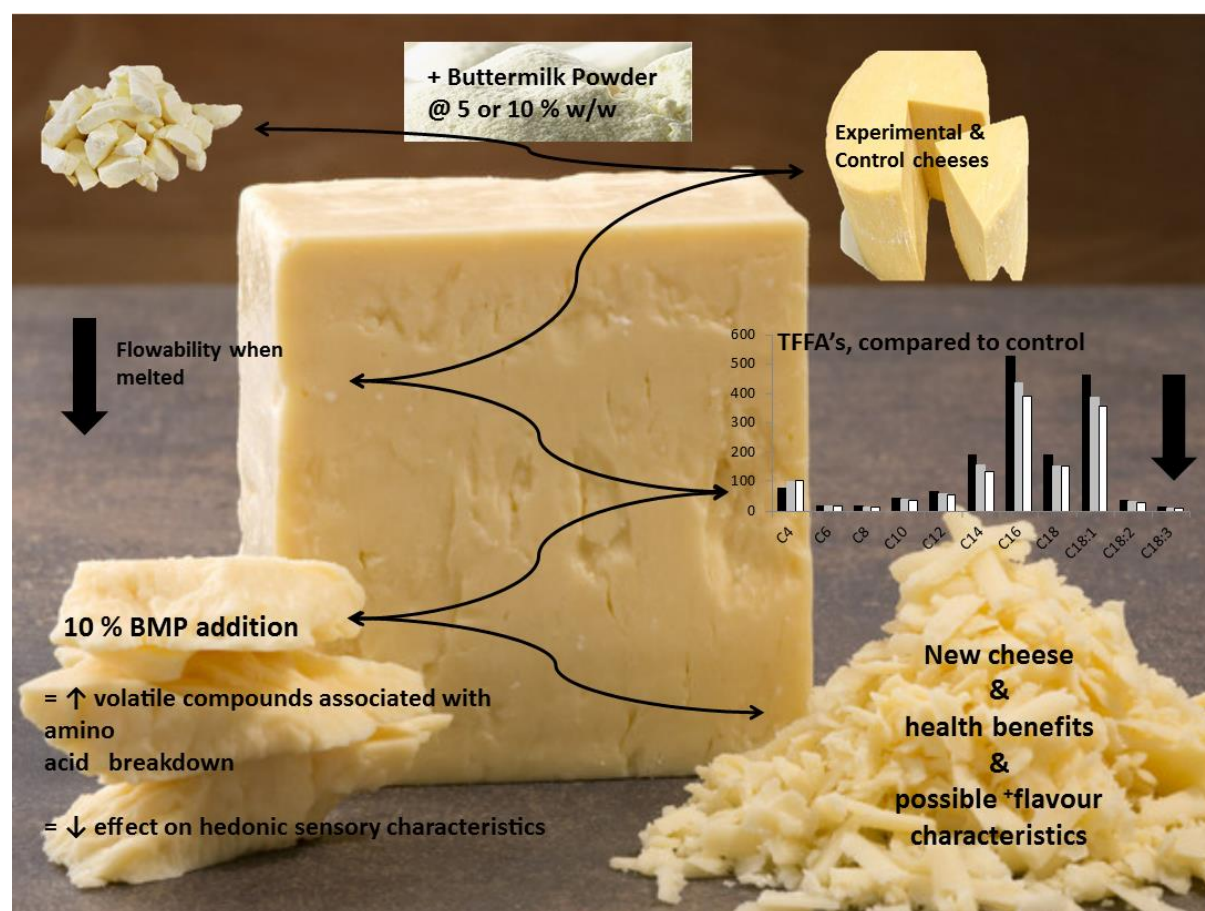
**Table 3:** Sensory Affective Hedonic Evaluation (grey) and Ranking descriptive analysis (white) of experimental Cheddar- type cheese samples

|                              | Control cheese                 | BM cheese           | 5 % BMP cheese      | 10 % BMP cheese     |
|------------------------------|--------------------------------|---------------------|---------------------|---------------------|
| <sup>1</sup> <i>Hedonics</i> |                                |                     |                     |                     |
| Liking of appearance         | <sup>3</sup> 6.80 <sup>a</sup> | 6.26 <sup>b</sup>   | 6.53 <sup>a,b</sup> | 6.40 <sup>a,b</sup> |
| Liking of aroma              | 6.78 <sup>a</sup>              | 5.88 <sup>b</sup>   | 5.70 <sup>b</sup>   | 6.31 <sup>a</sup>   |
| Liking of flavour            | 6.40 <sup>a</sup>              | 5.40 <sup>b</sup>   | 5.55 <sup>b</sup>   | 5.84 <sup>a,b</sup> |
| Liking of texture            | 6.25 <sup>a</sup>              | 5.66 <sup>a,b</sup> | 5.54 <sup>b</sup>   | 5.70 <sup>a,b</sup> |
| Overall acceptability        | 6.46 <sup>a</sup>              | 5.80 <sup>b</sup>   | 5.48 <sup>b</sup>   | 5.73 <sup>b</sup>   |
| <sup>2</sup> <i>RDA</i>      |                                |                     |                     |                     |
| Colour                       | 4.94 <sup>a</sup>              | 4.64 <sup>a</sup>   | 5.16 <sup>a</sup>   | 5.06 <sup>a</sup>   |
| Creamy aroma                 | 5.36 <sup>a</sup>              | 5.05 <sup>a,b</sup> | 4.69 <sup>b</sup>   | 4.85 <sup>a,b</sup> |
| Sweaty/sour aroma            | 5.04 <sup>c</sup>              | 5.85 <sup>a</sup>   | 5.28 <sup>b</sup>   | 5.06 <sup>c</sup>   |
| Oxidised aroma               | 1.58 <sup>a</sup>              | 1.67 <sup>a</sup>   | 1.25 <sup>a</sup>   | 1.56 <sup>a</sup>   |
| Barnyard aroma               | 1.93 <sup>a,b</sup>            | 2.56 <sup>a</sup>   | 1.85 <sup>a,b</sup> | 1.48 <sup>b</sup>   |
| Firmness in mouth            | 5.97 <sup>a</sup>              | 4.76 <sup>c</sup>   | 5.66 <sup>b</sup>   | 5.47 <sup>b</sup>   |
| Pasty texture                | 4.66 <sup>a</sup>              | 4.09 <sup>a,b</sup> | 3.63 <sup>b</sup>   | 3.96 <sup>a,b</sup> |
| Crumbly texture              | 3.86 <sup>b</sup>              | 3.60 <sup>c</sup>   | 4.64 <sup>a,b</sup> | 5.09 <sup>a</sup>   |
| Sweet taste                  | 4.07 <sup>a,b</sup>            | 3.29 <sup>b</sup>   | 3.86 <sup>a,b</sup> | 4.60 <sup>a</sup>   |
| Salt taste                   | 4.80 <sup>a</sup>              | 4.95 <sup>a</sup>   | 4.39 <sup>a</sup>   | 4.28 <sup>a</sup>   |
| Sour taste                   | 3.93 <sup>b</sup>              | 5.81 <sup>a</sup>   | 4.39 <sup>b,c</sup> | 3.45 <sup>c</sup>   |
| Bitter taste                 | 2.57 <sup>b</sup>              | 3.94 <sup>a</sup>   | 2.77 <sup>b</sup>   | 2.57 <sup>b</sup>   |
| Cream flavour                | 5.35 <sup>a</sup>              | 4.74 <sup>b,c</sup> | 5.25 <sup>a,b</sup> | 4.37 <sup>c</sup>   |
| Cheddar flavour              | 5.78 <sup>a</sup>              | 5.20 <sup>a,b</sup> | 5.43 <sup>a,b</sup> | 4.84 <sup>b</sup>   |
| Dairy sweet flavour          | 4.50 <sup>a</sup>              | 3.02 <sup>b</sup>   | 3.70 <sup>b</sup>   | 4.50 <sup>a</sup>   |
| Dairy fat flavour            | 4.61 <sup>a</sup>              | 3.70 <sup>b</sup>   | 4.29 <sup>a,b</sup> | 3.97 <sup>a,b</sup> |
| Fruity estery flavour        | 2.71 <sup>a,b</sup>            | 2.37 <sup>c</sup>   | 2.53 <sup>b,c</sup> | 3.43 <sup>a</sup>   |
| Off flavour                  | 1.07 <sup>b</sup>              | 2.22 <sup>a</sup>   | 1.43 <sup>a,b</sup> | 1.21 <sup>b</sup>   |
| Oxidised flavour             | 0.92 <sup>c</sup>              | 1.95 <sup>a</sup>   | 1.39 <sup>b,c</sup> | 1.23 <sup>b,c</sup> |
| Barnyard off-flavour         | 1.22 <sup>b</sup>              | 1.87 <sup>a</sup>   | 1.58 <sup>b</sup>   | 1.27 <sup>b</sup>   |

<sup>1</sup> Mean data of 27 naïve assessors. <sup>2</sup> Relative descriptive analysis. Data is the means of 8 trained assessors.

<sup>3</sup> (a-c) Means within a row with different superscripts differ significantly ( $P < 0.05$ ). Values presented are the means of three replicate trials (cheeses were manufactured in triplicate). Statistical analysis via Tukey based ANOVA.

Graphical abstract



## Highlights

- Buttermilk (BM) addition to cheese milk resulted in significantly higher levels of Free fatty acids, Buttermilk powder (BMP) addition significantly reduced FFA levels.
- BMP addition increased volatile compounds associated with amino acid breakdown; BM addition increased volatile compounds originating from lipolysis.
- BMP addition resulted in a similar sensory profile to the control cheese compared to the BM cheese
- BM addition scored higher for off-flavours and oxidized flavours compared to all other cheese, while 10 % BMP addition was comparable to control for majority of flavor aspects.